Micro and Nanotechnology; Nanopores II

2548-Pos Board B534
Analysis of ion Channel Activities in Lipid Bilayers Suspended Over Microwells on SI Substrates
Koji Sumitomo, Arianna McAllister, Yukihiro Tamba, Youichi Shinozaki, Keiichi Torimitsu.
One of the key techniques to fabricate a nanobio device that works with functioning membrane proteins is the creation of stable lipid bilayers, guaranteeing protein activity and the detection of their functions. In order to develop the measurement system of ion channel activities, here, suspended lipid bilayers were formed over the microwells fabricated on the Si substrate. Giant unilamellar vesicles (GUVs) with and without gramicidin A (gA) were ruptured over the microwells and the pH indicator pyranine was confined in the microwells by a lipid bilayer. Activity of gA ion channels contained in the suspended lipid bilayer was analyzed by the change of fluorescent intensity of pyranine.
Increasing the pH outside of the microwells by exchanging solution led to an increase in the fluorescent emission of pyranine confined in the microwells. Sealing of the micro-wells by the lipid bilayer was good enough such that no leakage of pyranine was observed during functional analysis. A clear difference in the rate of fluorescent intensity changes is seen between lipid bilayers with and without gA ion channels, indicating that transport of protons through gA ion channels was observed. Proton leak through the lipid membrane was estimated to be $10 \times 10^{-6} \sim 10 \times 10^{-5}$ cm/sec.
This successful creation of stable lipid bilayers on Si chips with peptides as biological signal transducers is an important step towards the development of large and versatile sensor arrays which allow parallel single channel recordings.

2549-Pos Board B535
Dehydration and Ionic Conductance Quantization in Nanopores
Michael Zwolak, James Wilson, Johan Lagerqvist, Massimiliano Di Ventra.
We study ion transport through nanopores via molecular dynamics calculations. Due to the confined geometry and large local field of a single ion, the nanoscale atomic configurations of species influence the ionic conductance [1]. In particular, hydration layers that form around ions in aqueous solution create a series of energy barriers to ion transport. As an ion enters the pore, these hydration layers have to be partially broken due to steric restrictions of the pore. The breaking of the layers proceeds in a highly nonlinear, step-like fashion, giving rise to a strong nonlinear dependence of the electrostatic energy barrier on the pore diameter and therefore also a step-like conductance. We discuss this effect as well as the conditions under which it may be experimentally observed [1,2].
[1] M. Zwolak, J. Lagerqvist, and M. Di Ventra, Phys. Rev. Lett. 103, 128102 (2009)
[2] M. Zwolak, J. Wilson, and M. Di Ventra, arXiv:1005.2550, to appear J. Phys. Cond. Mat.

2550-Pos Board B536
Translocation of Biopolymers Through Protein Nanopores: Mechanistic Insights from MD Simulations
Andrew T. Guy, Syma Khalid.
The translocation of biopolymers through nanopores is a ubiquitous process in biology. In recent years, inspired by biology, some of these processes have been manipulated for applications in bionanotechnology, e.g. as stochastic sensors and potential DNA sequencing devices. To date research into such protein nanopores has largely focused on α-hemolysin (αHL), a transmembrane exotoxin from S. aureus. In the present study, we have developed simplified models of the wildtype αHL pore and its mutants, in order to study the translocation dynamics of DNA and peptides under the influence of an applied electric field. We show that interactions between rings of cationic amino acids and DNA backbone phosphates result in meta-stable tethering of nucleic acid molecules within the pore, leading us to propose a ''binding and sliding'' mechanism for translocation. We also observe folding of DNA into non-linear conformational intermediates during passage through the confined nanopore environment, helping to rationalize experimentally determined trends in residual current and translocation efficiency for αHL and its mutants. Finally, we explore the translocation of peptides (both helical and extended) through our model pores as a model of protein transport.

2551-Pos Board B537
Development and In Vivo Application of a Novel Family of Dendrimer-Based Fluorescent Biosensensors
Lorenzo Albertazzi, Barbara Storti, Laura Marchetti, Fabio Beltram.
Fluorescent sensors are powerful tools to unveil dynamical changes of ions or biomolecules concentration in vivo. Both Green Fluorescent Proteins (GFPs) and fluorescent dyes were employed to measure biological relevant species. Synthetic fluorophores were largely used to measure a wide range of biological relevant species such as pH, Ca2+, K+, Cl-. However these molecules suffer of several limitations: i)difficulties in intracellular targeting; ii)no ratiometric imaging allowed; iii)poor solubility; iv)time dependent cell leakage.
To overcome some limitations of fluorescent biosensors we developed a nanosized platform able to carry multiple copies of different dyes inside living cells. Synthesis and targeted intracellular delivery of ratiometric fluorescent pH sensors is reported.
We choose dendrimers as scaffold for this new family of reporters. Dendrimers are multifunctional hyper branched polymers recently applied in sensing and drug delivery thanks to their interesting features such as multifunctionality, tunable chemical properties and low toxicity.
Ratiometric pH sensors were realized by conjugating dendrimers to a pH sensitive dye and a pH insensitive dye in order to allow signal normalization for sensor concentration. Different sensors with tuned affinity for H+ were developed demonstrating the general applicability of this modular approach.
Dendrimer-based sensors were calibrated in vitro and then applied to living cells. We demonstrate the ability of the dendritic carrier to selectively deliver the sensing moieties to different intracellular compartments such as cytoplasm, nucleus, nucleoli, lysosomes and plasma membrane. Confocal microscopy was employed to acquire targeted pH maps with high spatial resolution in many cell lines. In particular the sensor was successfully used to detect dynamic changes of pH in different organelles.
In conclusion with our work we proposed a new family of fluorescent reporters with improved properties for the investigation of a number of biological processes.

2552-Pos Board B538
Red Blood Cell Sickling in Microdroplet Arrays
Paul Abbyad, Remi Dangla, Pierre-Louis Tharaux, Charles Baroud, Antigoni Alexandrou.
We have developed a microfluidic approach to study the sickling of red blood cells associated with sickle cell anemia by varying the oxygen partial pressure within arrays of microdroplets in flowing oil. By etching holes into the channel's top surface, droplets can be held stationary while maintaining an external flow of oil. Such control is possible for drops that are squeezed by the channel roof, by allowing them to reduce their surface energy as they enter into a local depression. By using the perfluorinated carrier oil as a sink or source of oxygen, the oxygen level within the water droplets equilibrates through exchange with the surrounding oil. This provides control over the oxygen partial pressure within an aqueous microdroplet ranging from 1 kPa to ambient partial pressure, i.e. 21 kPa. The controlled deoxygenation is used to trigger the polymerization of hemoglobin within sickle red blood cells, encapsulated within drops. This process is observed using polarization microscopy, which yields a robust criterion to detect polymerization based on transmitted light intensity through crossed polarizers (Abbyad et al., Lab Chip, 2010, 10, 2513). We demonstrate in particular how the oxygen levels within the drops can be controlled spatially and temporally, either by exposing rows of drops to two streams of oil at different gas concentrations or by periodically switching oil inputs to vary the gas concentration of drops as a function of time. Cycles of oxygenation and deoxygenation of anchored droplets induce depolymerization and polymerization of the hemoglobin, thus providing a method to simulate the cycling that takes place in physiological blood flow. Droplet content is varied to study the effect of different biochemical or therapeutic agents on sickling.

2553-Pos Board B539
Spectrally Selective Light Induced Release from Gold Coated Liposomes
Sarah Leung, Marek Romanowski.
In the interest of creating functional nanostructures to address unmet medical needs for controlled delivery and release, we recently presented liposome-supported plasmon resonant gold nanoshells (Troutman et al., *Adv. Mater.* 2008, 20, 2604-2608). These composite structures are degradable into components of a size compatible with renal clearance, potentially enabling their use as multifunctional agents in imaging, diagnostic, therapeutic, and drug delivery applications. The liposome allows for the encapsulation of substances, including diagnostic and therapeutic agents, while the plasmon resonant structure facilitates the rapid release of encapsulated contents upon laser light illumination using a wavelength corresponding to the resonance band. Furthermore, the resonance of these gold-coated liposomes is tunable in the near-infrared range. Using this tunability, we achieved spectrally selective content release using two laser wavelengths, in which full content release occurs for liposomes spectrally matched with the illumination source and minimal release occurs for liposomes not matched with the source. Also, spectrally selective release is accomplished through the use of multiple, low intensity laser pulses, ensuring that illumination affects only the gold-coated liposomes and avoids heating the surrounding